Great Lakes
Chemical Corporation

8EHQ-0597-13930

P.O. BOX 2200 • ONE GREAT LAKES BOULEVARD • WEST LAFAYETTE, IN 47906 • PHONE: 317-497-6100 • FAX: 317-497-6123

2 May 1997

Document Control Office (7404)
Office of toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

Contains No CBI

RE: TSCA Section 8(e) Notification on Acetylfuran
(When responding, please refer to JAB-97-101)

ATTN: TSCA Section 8(e) Coordinator

Great Lakes Chemical Corporation is submitting a Section 8(e) substantial risk notification regarding an acute inhalation toxicity study in rats with 2-acetylfuran; CAS Registry Number 1192-62-7. The following information was received for this methyl-heteocyclic ketone on April 25, 1997 via a final report from our overseas operation in the United Kingdom. A copy of the final report is enclosed.

The test article was administered by inhalation at chamber atmospheric mean concentrations of 0.89, 1.76, or 2.32 mg/L. Length of exposure to the vapour generated atmospheres was four (4) hours using a nose-only exposure system. The number of rats used per exposure group was five males and five females of the Sprague Dawley CD strain. In addition to the three four-hour exposure groups, a group of five males and five females were exposed to the mean concentration of 2.38 mg/L for one hour. The animals were observed for mortality and overt signs of toxicity at 30 minutes and/or hourly intervals during the exposure, one hour after exposure termination, and subsequently once daily for the remainder of the 14-day study. The observation period was extended to 21 days for the surviving animals exposed to 2.38 and 2.32 mg/L. Individual body weights were recorded on study days 0, 7, and 14 or at time of death. Surviving animals that were extended to 21 days of observation were also weighted on Day 21. All animals were subjected to necropsy and a detailed macroscopic examination.

Common clinical signs of toxicity during the study included hunched posture, pilo-erection, gasping, laboured and noisy respiration, increased or decreased respiratory rate, and red/brown staining around the eyes, nose, mouth, and ano-genital region. Other findings noted during the study were wet fur, occasional sneezing, and red/brown staining of the fur. Noted as systemic signs of toxicity were lethargy, ptosis, pallor of the

extremities, dehydration and distended abdomen. Also observed were occasional/isolated incidents of ataxia, chronic convulsions, tiptoe gait, chromodacryorrhoea, nasal discharge, increased salivation, and corneal opacity. A number of these noted signs of toxicity persisted throughout the study in all dose groups and were considered severe enough to extend the observation period for another seven days (Day 21) of the surviving animals exposed at 2.38 and 2.32 mg/L.

Bodyweight loss was observed in surviving animals from all dose groups during week one (1) of the study. During week two (2), body weight gains had generally recovered. There were, however, negligible gains noted in isolated females exposed at 2.38 mg/L and 2.32 mg/L. Bodyweight gains in all other surviving animals exhibited recovery by either the end of the 14- or 21-day study period.

Animals that expired or were sacrificed in extremis during the study had lung findings that included swelling, redness, pallor, and dark patches. Liver, spleen, and kidney changes were also noted with similar findings that included darkening and pallor. Evidence of congestion, gaseous distention, and reddening in the gastro-intestinal tract was noted as well.

Under the conditions of this study, the acute inhalation median lethal concentration (LC_{50}) for a one-hour exposure was reported to be greater than 2.38 mg/L, the maximum attainable concentration. The acute inhalation median lethal concentration (LC_{50}) for a four-hours exposure was calculated to be 1.44 mg/L for male rats and 1.13 mg/L for female rats.

If you have any questions, please feel free to contact me at (765) 497-6223.

Sincerely,

John A. Biesemeier
Regulatory Toxicologist
Regulatory Affairs

JAB/clw

Enclosure

2-ACETYLFURAN:

ACUTE INHALATION TOXICITY

(NOSE ONLY) STUDY IN THE RAT

SPL PROJECT NUMBER: 541/019

AUTHOR: S M Blagden

STUDY SPONSOR:

Great Lakes Fine Chemicals Limited
Halebank
WIDNES
Cheshire
WA8 8NS

ISSUED BY:

Safepharm Laboratories Limited
P.O. Box No. 45
DERBY
DE1 2BT
UK

Telephone: (01332) 792896

Facsimile: (01332) 799018

SPL PROJECT NUMBER: 541/019

QUALITY ASSURANCE REPORT

The routine inspection of short term studies at Safepharm is carried out as a continuous process designed to encompass all major phases of each study type once per month. Dates of relevant monthly inspections are given below.

Date(s) of Inspection and Reporting:

09 December 1996

This report has been audited by Safepharm Quality Assurance Unit. It is considered to be an accurate account of the data generated and of the procedures followed.

Date of Report Audit:

30 January 1997

..... DATE: 16 APR 1997

J R Pateman BBiol MIBiol

For Safepharm Quality Assurance Unit

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that the objectives laid down in the protocol were achieved and as nothing occurred to adversely affect the quality or integrity of the study, I consider the data generated to be valid. This report fully and accurately reflects the procedures used and data generated.

The work described was performed in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health 1989). These Principles are in accordance with GLP standards published as OECD Environment Monograph No. 45 (OCDE/GD(92)32); and are in conformity with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

These international standards are acceptable to the United States Environmental Protection Agency and Food and Drug Administration, and fulfil the requirements of 40 CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58 (as amended).

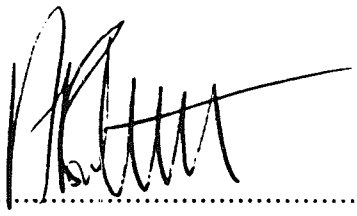
..... DATE: 14 APR 1997

S M Blagden FIAT
Study Director
for Safepharm Laboratories

SPL PROJECT NUMBER: 541/019

AUTHENTICATION

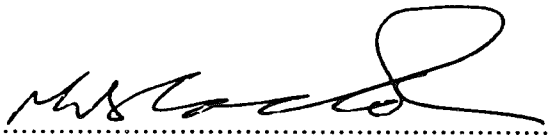
I, the undersigned, hereby declare that the analytical data presented in this report were compiled by me or under my supervision and that the results reported herein accurately reflect the data obtained.



..... DATE: 15 APR 1997

A J Bartlett CChem MRSC
Head of Analytical Chemistry

Approved for issue:



..... DATE: 14 APR 1997

M P Blackwell BSc (Hons) FIAT
Head of Repeat Dose and Inhalation Toxicology

CONTENTS

CONTENTS (continued)

SPL PROJECT NUMBER: 541/019

SUMMARY

STUDY SPONSOR	:	GREAT LAKES FINE CHEMICALS LIMITED
STUDY TITLE	:	ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT
TEST MATERIAL	:	2-ACETYLFURAN

1. A study was performed to assess the acute inhalation toxicity of the test material, as supplied, by exposing four groups of ten Sprague-Dawley CD strain rats (five males and five females) to various concentrations of a vapour atmosphere. One group of animals was exposed for one hour and three groups of animals were exposed for four hours using a nose only exposure system.

   The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 403 "Acute Inhalation Toxicity" referenced as Method B2 in Commission Directive 92/69/EEC "Acute Toxicity - Inhalation" (which constitutes Annex V of Council Directive 67/548/EEC).

2. The mean achieved atmosphere concentrations (determined analytically) were as follows:

	ATMOSPHERE CONCENTRATION		
GROUP NUMBER	MEAN ACHIEVED mg/l	STANDARD DEVIATION	NOMINAL mg/l
1*	2.38	0.44	11.5
2#	2.32	0.38	11.5
4#	1.76	0.16	3.8
3#	0.89	0.08	1.5

* = one hour exposure # = four hour exposure

3. The mortality data were summarised as follows:

GROUP NUMBER	MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	DEATHS		
		MALE	FEMALE	TOTAL
1*	2.38	1/5	0/5	1/10
2#	2.32	4/5	3/5	7/10
4#	1.76	3/5	4/5	7/10
3#	0.89	0/5	1/5	1/10

* = one hour exposure # = four hour exposure

4. **Clinical Observations**

Common abnormalities noted during the study included wet fur, hunched posture, pilo-erection, gasping, laboured and noisy respiration, increased or decreased respiratory rate, occasional sneezing, red/brown staining around the eyes, snout, mouth and ano-genital region and red/brown staining of the fur. Signs of lethargy, ptosis, pallor of the extremities, dehydration and distended abdomen were also noted and there were occasional or isolated incidents of ataxia, clonic convulsions, tiptoe gait, chromodacryorrhoea, nasal discharge, increased salivation and corneal opacity. Signs of toxicity persisted throughout the study in all dose groups. The severity of those seen on Day 14 in animals exposed to 2.38 mg/l for one hour or 2.32 mg/l for four hours was such that the observation period was extended to twenty-one days. On Day 21 several animals from these two dose groups still showed clinical abnormalities; signs of hunched posture, pilo-erection, decreased respiratory rate, laboured respiration, occasional sneezing and red/brown staining around snout were noted in two surviving animals exposed for four hours while in those exposed for one hour abnormalities were confined to occasional sneezing and an incident of red/brown staining around the snout.

5. **Bodyweight**

Bodyweight loss or reduced bodyweight gain were observed in surviving animals from all dose groups during Week 1 of the study. During Week 2 bodyweight gain had generally recovered but negligible bodyweight gain was noted in isolated females exposed to 2.38 mg/l (one hour), 2.32 mg/l (four hours) or 0.89 mg/l.

Bodyweight gain in all other surviving animals recovered by the end of the fourteen or twenty-one day study period.

6. **Necropsy**

The animals that died or were killed *in extremis* during the study commonly showed lung abnormalities at necropsy and these included swelling, abnormal redness, pallor and dark patches. Liver changes were noted and included darkening, pallor and patchy pallor. Incidents of small or pale spleen and darkening or pallor of the kidneys were also noted and there was evidence of congestion, gaseous distension and reddening in the gastro-intestinal tract. Two surviving animals exposed for one hour to 2.38 mg/l showed dark patches on the lungs and one female showed an enlarged and hardened lobe of the liver. No other abnormalities were detected in surviving animals at the end of the study.

7. **Conclusion**

The acute inhalation median lethal concentration for one hour exposure (LC_{50} 1h) to the test material 2-ACETYLFURAN, in the Sprague-Dawley CD strain rat, was considered to be greater than 2.38 mg/l which was the maximum attainable concentration.

The acute inhalation median lethal concentration for four hours exposure (LC_{50} 4h) and 95% confidence limits of the test material 2-ACETYLFURAN, in the Sprague-Dawley CD strain rat, were calculated to be:

All animals	:	1.33 (1.00 - 1.75) mg/l
Males only	:	1.44 (1.05 - 1.97) mg/l
Females only	:	1.13 (0.57 - 2.23) mg/l

2-ACETYLFURAN:
ACUTE INHALATION TOXICITY
(NOSE ONLY) STUDY IN THE RAT

1. INTRODUCTION

The study was designed to assess the acute inhalation toxicity of the test material in the Sprague-Dawley CD strain rat in compliance with the recommendations of the OECD Guidelines for Testing of Chemicals (1981) No. 403 "Acute Inhalation Toxicity" referenced as Method B2 in Commission Directive 92/69/EEC "Acute Toxicity - Inhalation" (which constitutes Annex V of Council Directive 67/548/EEC).

The test system was chosen because the rat has been shown to be a suitable model for this type of study and is recommended in the test method. The results of the study are believed to be of value in predicting the likely toxicity of the test material to man.

The study was performed between 6 September 1996 and 10 December 1996.

2. TEST MATERIAL

2.1 Description, Identification and Storage Conditions

Sponsor's identification	:	2-ACETYLFURAN
Chemical name	:	2-ACETYLFURAN
Batch number	:	95E16
Date received	:	8 August 1996
Description	:	light brown crystalline block
Storage conditions	:	room temperature

Data relating to the identity, purity and stability of the test material are the responsibility of the Sponsor. A Certificate of Analysis was supplied by the Sponsor and is included in Appendix VII, which gives the purity of the test material at 99.7%.

3. METHODS

3.1 Animals and Animal Husbandry

Twenty male and twenty female young adult Sprague-Dawley CD strain rats were supplied by Charles River (UK) Ltd, Margate, Kent. At the start of the study the animals were approximately eight to ten weeks old, the males weighed 231 to 310g, and the females 201 to 263g. After an acclimatisation period of at least five days the animals were selected at random and given a number unique within the study by ear punching and a number written on a colour coded cage card.

The animals were housed in groups of five by sex in solid-floor polypropylene cages with stainless steel lids, furnished with softwood flakes (Datesand Ltd, Cheshire, UK). With the exception of the exposure period, free access to mains drinking water and food (Rat and Mouse Expanded Diet No. 1, Special Diets Services Limited, Witham, Essex, UK) was allowed throughout the study.

Temperature and humidity were maintained within the target ranges of 21 ± 2°C and 55 ± 15% respectively. The rate of air exchange was at least fifteen changes per hour and the lighting was controlled by a time switch to give twelve hours continuous light and twelve hours darkness. The animals were retained in this accommodation at all times except during the exposure period.

3.2 Inhalation Exposure

3.2.1 Atmosphere Generation

The test material in its original container was placed in a water bath at a temperature not exceeding 40°C. Once all the test material had liquified a sufficient amount was transferred to a glass round-bottomed flask also placed in a water bath held at a constant temperature of 40°C. Filtered compressed air was forced through a glass sinter immersed in

the liquid test material and the resultant vapour was ducted into the top of the exposure chamber.

Compressed air was supplied by means of an oil free compressor and was passed through a water trap and respiratory quality filters before it was introduced to the flask.

The cylindrical exposure chamber had a volume of 30 litres ± 1 litre. The concentration within the exposure chamber was controlled by adjusting the air flow rate through the test material. The extract from the exposure chamber passed through a 'scrubber' trap and was connected with a high efficiency filter to a metered exhaust system. A schematic diagram of the dynamic (continuous flow) system employed is shown in Figure I. The chamber was maintained under negative pressure.

3.2.2 Test Atmosphere Characterisation

Prior to the start of the study, test material atmospheres were generated within the exposure chamber. During this characterisation period air flow settings, test material input and the sampling system were varied to achieve the required atmospheric concentrations. Particle size analysis was performed several times using a Cascade impactor with back up solvent trap to determine whether the material was condensing within the chamber.

3.2.3 Pre-Study Sighting

During characterisation groups of one or two rats were exposed to varying atmosphere concentrations of the vapour material. Severe adverse effects were noted at a near-maximum attainable concentration but there were no deaths.

3.2.4 Exposure Procedure

Each rat was individually held in a tapered, polycarbonate restraining tube fitted onto a single tier of the exposure chamber and sealed by means of a rubber 'O' ring. Only the nose of each animal was exposed to the test atmosphere.

Four groups, each of ten rats (five males and five females) were subjected to a single exposure to the test material. The first two groups of animals were exposed simultaneously to the maximum attainable concentration. One group was exposed for one hour and one group was exposed for four hours. Further concentrations were selected after consideration of the results of the previous exposure.

3.2.5 Exposure Chamber Temperature and Relative Humidity

The temperature and relative humidity inside the exposure chamber were measured by an electronic thermometer/humidity meter (Kane-May Ltd, Welwyn Garden City, Hertfordshire, UK) located in a vacant port in the animals' breathing zone of the chamber and recorded every thirty minutes throughout each exposure period. Individual values are given in Appendices I and II.

3.2.6 Exposure Chamber Oxygen Concentrations

Oxygen levels within the exposure chamber were measured by an electronic oxygen analyser (Servomex (UK) Ltd, Crowborough, East Sussex) located in a sampling port in the animals' breathing zone during each exposure period. The test atmosphere was generated to contain at least 19% oxygen. Individual values are given in Appendix III.

3.2.7 Exposure Chamber Atmosphere Concentration

The chamber concentration was sampled between 15 and 28 minutes after the start of exposure and at approximately hourly intervals during each exposure period.

The sampling procedure involved pumping three litres of the chamber atmosphere through a glass impinger containing 40 ml of acetonitrile. After sampling the dreschel head was flushed through with a further 10 ml of acetonitrile to remove any deposits. This gave a 50 ml sample to be submitted for chemical analysis. The method of analysis is given in Appendix VI.

The nominal chamber concentration was calculated as follows:

$$\text{Nominal concentration (mg/l)} = \frac{\text{Weight of test material used (mg)}}{\text{Total air flow through chamber (l)}}$$

3.2.8 Particle Size Distribution

The particle size of the generated atmosphere of the test material inside the exposure chamber was determined several times during characterisation and once during each exposure period using a Cascade Impactor. This device consisted of six impactor stages with stainless steel collection substrates (10, 6, 3.5, 1.6, 0.9 and 0.5 μm cut-off points), a back up glass fibre filter housed in an aluminium sampler and a back up solvent trap containing 40 ml of acetonitrile. The sampler was temporarily sealed in a sampling port in the animals' breathing zone. Exposure chamber air was drawn through the Cascade Impactor using a vacuum pump for a suitable time period.

The collection substrates were weighed before and after sampling. The back up solvent trap was flushed through with a further 10 ml of acetonitrile to give a 50 ml sample which was submitted for chemical

analysis. The method of analysis, given in Appendix VI, was identical to that used for the atmosphere concentration samples.

3.3 Observations

3.3.1 Clinical Signs

Animals exposed to 2.38 mg/l or 2.32 mg/l were observed, for clinical signs, after 30 minutes exposure. Then animals from all groups were observed at hourly intervals during the exposure and/or immediately on removal from the restraining tubes at the end of the exposure, one hour after termination of the exposure and subsequently once daily for fourteen days. The observation period was extended to twenty-one days for the surviving animals exposed to 2.38 mg/l or 2.32 mg/l since marked signs of toxicity were still evident on Day 14. Any deaths or evidence of overt toxicity were recorded at each observation.

3.3.2 Bodyweight

Individual bodyweights were recorded prior to treatment on the day of exposure and on Days 7 and 14 or at death. Surviving animals exposed to 2.38 mg/l or 2.32 mg/l were also weighed on Day 21.

3.3.3 Necropsy

At the end of the fourteen or twenty-one day observation period, the surviving animals were killed by intravenous overdose of sodium pentobarbitone. All animals, including those that died or were killed *in extremis* during the study, were subjected to a full external and internal examination, and any macroscopic abnormalities were recorded. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritancy or local toxicity.

3.4 Evaluation of Data

Data evaluations included the relationship, if any, between the animals' exposure to the test material and the incidence and severity of all abnormalities including behavioural and clinical observations, necropsy findings, bodyweight changes, mortality and any other toxicological effects.

Using the mortality data obtained, the acute inhalation median lethal concentration (LC_{50}) of the test material was estimated for one hour and four hours exposure. The LC_{50} and 95% confidence limits were calculated for the four hour exposure using the method of Thompson W R (1947).

4. ARCHIVES

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archives for a period of five years. After this period, the Sponsor's instructions will be sought.

5. RESULTS

5.1 Exposure Chamber Concentration

The actual concentration of the test material was measured by a validated gas chromatography method at regular intervals during the exposure period. The mean values obtained were:

ATMOSPHERE CONCENTRATION			
GROUP NUMBER	MEAN ACHIEVED mg/l	STANDARD DEVIATION	NOMINAL mg/l
1*	2.38	0.44	11.5
2#	2.32	0.38	11.5
4#	1.76	0.16	3.8
3#	0.89	0.08	1.5

* = one hour exposure # = four hour exposure

The exposure chamber concentrations and chamber flow rates are given in Tables 1 to 3.

Chamber air flow rates were maintained by vacuumed exhaust at 18 l/min providing 36 air changes per hour.

Theoretical chamber equilibration times (T_{99}) (Silver, 1946) were calculated to be 8 minutes for each dose group but, to ensure stable atmospheres at the start of exposure, the atmospheres were generated for at least 16 minutes prior to the introduction of animals to the chamber.

5.2 Particle Size Distribution

The results of the particle size analysis performed during characterisation and during each exposure showed no test material present on the impaction plates and an insignificant amount on the back up filter but a substantial amount in the solvent trap. This suggests that the atmosphere sampled was entirely in the vapour phase as it passed through the impactor.

5.3 Mortality Data

The mortality data are given in Table 5 and are summarised as follows:

GROUP NUMBER	MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	DEATHS		
		MALE	FEMALE	TOTAL
1*	2.38	1/5	0/5	1/10
2#	2.32	4/5	3/5	7/10
4#	1.76	3/5	4/5	7/10
3#	0.89	0/5	1/5	1/10

* = one hour exposure # = four hour exposure

Following a one hour exposure of a group of ten rats to 2.38 mg/l, one male was killed *in extremis* on Day 3.

Following four hours exposure of a group of ten rats to 2.32 mg/l two males and one female died and two males and one female were killed *in extremis* on Day 2. A third female was killed *in extremis* on Day 3. One male and two females survived.

Exposure to 1.76 mg/l also resulted in seven deaths; one female was found dead on Day 2, one male and two females were found dead on Day 3, one male and one female were killed *in extremis* on Day 3 and one male was found dead on Day 4. Two males and one female survived.

One female exposed to 0.89 mg/l was killed *in extremis* on Day 2.

5.4 Clinical Observations

Individual clinical observations are given in Tables 6 to 9.

During exposure animals from all dose groups showed signs of wet fur and increased or decreased respiratory rate.

On removal from the chamber the animals exposed for one hour to 2.38 mg/l commonly showed wet fur, hunched posture, pilo-erection, increased or decreased respiratory rate, ptosis and isolated incidents of noisy respiration and red/brown staining around eyes. One hour after completion of exposure wet fur was no longer evident but there were additional signs of gasping, laboured and noisy respiration and occasional sneezing.

The animals exposed to 2.32 mg/l for four hours similarly showed wet fur, hunched posture, pilo-erection, ptosis, increased or decreased respiratory rate and laboured and noisy respiration on removal from the chamber. Several animals showed laboured respiration and ptosis was noted in one female. One hour after completion of exposure wet fur was no longer evident but the other symptoms persisted.

On Day 1 following exposure animals in these two dose groups commonly showed hunched posture, pilo-erection, laboured respiration and increased or decreased respiratory rate. There were incidents of gasping or noisy respiration, ptosis, pallor of the extremities, increased salivation, red/brown staining around the eyes, snout or mouth and red/brown staining of the fur.

Several animals in the four hour exposure group also appeared lethargic and there were signs of wet fur and chromodacryorrhoea. On Day 2 following exposure surviving animals in the four hour exposure group continued to show severe signs of toxicity together with additional incidents of dehydration and isolated incidents of clonic convulsions, nasal discharge and occasional sneezing. Three animals showed corneal opacity. The animals exposed for one hour also continued to show similar severe signs

together with occasional or isolated incidents of lethargy, dehydration, distended abdomen, tiptoe gait, occasional sneezing and red/brown staining around the ano-genital region. On Day 3 one animal in each group showed chromodacryorrhoea and/or gasping respiration.

The animals that survived in these two dose groups continued to show signs of toxicity up to the end of the normal fourteen day observation period. However, due to the animals' condition on Day 14 this period was extended for a further seven days. At the end of the study signs of hunched posture, pilo-erection, laboured respiration, decreased respiratory rate, occasional sneezing and red/brown staining around snout were still evident in two surviving animals in the four hour exposure group whilst in those exposed for one hour abnormalities were confined to occasional sneezing and an incident of red/brown staining around the snout.

On removal from the chamber following exposure to 1.76 mg/l all animals showed wet fur, hunched posture, pilo-erection, noisy respiration and increased or decreased respiratory rate. Incidents of laboured respiration and isolated signs of ataxia, pallor of the extremities and red/brown staining around the snout were noted. One hour after completion of exposure wet fur was no longer evident but there were signs of laboured respiration, ptosis and one female appeared lethargic. On Day 1 following exposure signs of toxicity were more marked and included additional signs of lethargy, laboured and gasping respiration, pallor of the extremities, distended abdomen, increased salivation and red/brown staining around the eyes, snout and mouth. On Day 2 the condition of several surviving animals had deteriorated and on Day 3 the condition of two of the remaining animals had deteriorated further. One of these animals showed corneal opacity. Severe signs of toxicity persisted for several days in the three survivors in this dose group and at the end of the study hunched posture, decreased respiratory rate and red/brown staining around the snout were still evident.

On removal from the chamber following exposure to 0.89 mg/l wet fur, hunched posture and pilo-erection were commonly observed and there were signs of increased respiratory rate, noisy respiration, ptosis and red/brown staining around the eyes or snout. One hour after completion of exposure clinical abnormalities were confined to hunched posture, pilo-erection, increased respiratory rate, noisy respiration and an incident of occasional sneezing. On Day 1 following exposure, however, laboured respiration and decreased respiratory rate were observed, occasional sneezing was more common, red/brown staining around the eyes or snout had recurred in two animals and one female showed red/brown staining around mouth. On Day 2 similar signs of toxicity were noted although one female additionally showed ataxia, gasping and noisy respiration, ptosis, pallor of the extremities and red/brown staining around the snout. Surviving animals in this dose group continued to show signs of toxicity for several days and these included isolated incidents of tiptoe gait and gasping respiration. Once again abnormalities persisted throughout the study although four animals recovered to appear normal between Days 6 and 14.

5.5 Bodyweight

Individual bodyweights are given in Tables 10 to 13.

Bodyweight loss or reduced bodyweight gain were observed in surviving animals from all dose groups during Week 1 of the study. During Week 2 bodyweight gain had generally recovered but negligible bodyweight gain was noted in isolated females exposed to 2.38 mg/l (one hour), 2.32 mg/l (four hours) or 0.89 mg/l.

All other surviving animals showed normal bodyweight gain by the end of the fourteen or twenty-one day study period.

5.6 Necropsy

Individual necropsy findings are given in Tables 14 to 17.

The animals that died or were killed *in extremis* during the study commonly showed lung abnormalities at necropsy and these included swelling, abnormal redness, pallor and dark patches. Liver changes were noted and included darkening, pallor and patchy pallor. Incidents of small or pale spleen and darkening or pallor of the kidneys were also noted and there was evidence of congestion, gaseous distension and reddening in the gastro-intestinal tract. Two surviving animals exposed for one hour to 2.38 mg/l showed dark patches on the lungs and one female showed an enlarged and hardened lobe of the liver. No other abnormalities were detected in surviving animals at the end of the study.

6. CONCLUSION

The acute inhalation median lethal concentration for one hour exposure (LC_{50} 1h) to the test material 2-ACETYLFURAN, in the Sprague-Dawley CD strain rat, was considered to be greater than 2.38 mg/l which was the maximum attainable concentration.

The acute inhalation median lethal concentration for four hours exposure (LC_{50} 4h) and 95% confidence limits of the test material 2-ACETYLFURAN, in the Sprague-Dawley CD strain rat, were calculated to be:

All animals	:	1.33 (1.00 - 1.75) mg/l
Males only	:	1.44 (1.05 - 1.97) mg/l
Females only	:	1.13 (0.57 - 2.23) mg/l

7. REFERENCES

Green J D *et al* (1984) Effect of Equilibration Zones on Stability, Uniformity and Homogeneity Profiles of Vapours and Aerosols in the ADG Nose Only Inhalation Exposure System. *Fundamental and Applied Toxicology* **4**, 768-777.

Silver S D (1946) Constant flow gassing chambers: Principles influencing design and operation. *J Lab Clin Med* **31**, 1153-1161.

Thompson W R (1947) Moving Average Interpolation. *Bact Reviews* **11**, 115-145.

TABLES

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 1

EXPOSURE CHAMBER ATMOSPHERE CONCENTRATIONS - DOSE GROUPS 1 AND 2

START OF SAMPLING DURING EXPOSURE (minutes)	CHAMBER FLOW RATE (l/min)	ATMOSPHERE CONCENTRATION (mg/l)
28	18	2.69
55	18	2.07
115	18	2.21
175	18	2.76
235	18	1.89
240	18	-

One hour exposure:

Mean achieved atmosphere concentration (mg/l) = 2.38

Standard Deviation = 0.44

Four hour exposure:

Mean achieved atmosphere concentration (mg/l) = 2.32

Standard Deviation = 0.38

- = not determined

$$\text{Nominal Concentration}^{*} = \frac{\text{Amount of material introduced (mg)}}{\text{Volume of air passed (l)}} = \frac{53900}{4680} = 11.5 \text{ mg/l}$$

* = Figures based on four hour exposure plus pre-exposure equilibration period of 20 minutes

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 2

EXPOSURE CHAMBER ATMOSPHERE CONCENTRATIONS - DOSE GROUP 4

START OF SAMPLING DURING EXPOSURE (minutes)	CHAMBER FLOW RATE (l/min)	ATMOSPHERE CONCENTRATION (mg/l)
15	18	1.78
55	18	1.56
116	18	1.64
176	18	1.87
235	18	1.94
240	18	-

Mean achieved atmosphere concentration (mg/l) = 1.76

Standard Deviation = 0.16

- = not determined

$$\text{Nominal Concentration}^{*} = \frac{\text{Amount of material introduced (mg)}}{\text{Volume of air passed (l)}} = \frac{18500}{4896} = 3.8 \text{ mg/l}$$

* = Figures based on four hour exposure plus pre-exposure equilibration period of 32 minutes

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 3

EXPOSURE CHAMBER ATMOSPHERE CONCENTRATIONS - DOSE GROUP 3

START OF SAMPLING DURING EXPOSURE (minutes)	CHAMBER FLOW RATE (l/min)	ATMOSPHERE CONCENTRATION (mg/l)
20	18	0.949
55	18	0.948
118	18	0.884
175	18	0.892
238	18	0.759
240	18	-

Mean achieved atmosphere concentration (mg/l) = 0.89

Standard Deviation = 0.08

- = not determined

$$\text{Nominal Concentration} * = \frac{\text{Amount of material introduced (mg)}}{\text{Volume of air passed (l)}} = \frac{7100}{4608} = 1.5 \text{ mg/l}$$

* = Figures based on four hour exposure plus pre-exposure equilibration period of 16 minutes

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 4

PARTICLE SIZE DISTRIBUTION

Impactor Stage	Cut off diameter (μm)	Amount collected (mg/l) During Dose Group:		
		2 (2.32 mg/l)	4 (1.76 mg/l)	3 (0.89 mg/l)
6	10.0	0.00	0.00	0.00
5	6.0	0.00	0.00	0.00
4	3.5	0.00	0.00	0.00
3	1.6	0.00	0.00	0.00
2	0.9	0.00	0.00	0.00
1#	0.5	0.02	0.00	0.00
Back up solvent trap	-	0.55	0.99	0.77

= values include amount collected on back up filter

- = not applicable

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 5

MORTALITY DATA

GROUP NUMBER	MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	SEX	DEATHS DURING EXPOSURE	DEATHS POST EXPOSURE (1 HOUR)	DEATHS DURING DAY OF OBSERVATION								TOTAL DEATHS
					1	2	3	4	5	6	7	8-14	
1*	2.38	Male	0	0	0	0	(1)	0	0	0	0	0▲	1/10
		Female	0	0	0	0	0	0	0	0	0	0▲	
2#	2.32	Male	0	0	0	4(2)	0	0	0	0	0	0▲	7/10
		Female	0	0	0	2(1)	(1)	0	0	0	0	0▲	
4#	1.76	Male	0	0	0	0	2(1)	1	0	0	0	0	7/10
		Female	0	0	0	1	3(1)	0	0	0	0	0	
3#	0.89	Male	0	0	0	0	0	0	0	0	0	0	1/10
		Female	0	0	0	(1)	0	0	0	0	0	0	

(n) = number of animals killed *in extremis*
* = one hour exposure

▲ = observation period extended to twenty-one days
= four hour exposure

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

KEY TO CLINICAL OBSERVATIONS

A	=	ataxia
Cc	=	clonic convulsions
Ch	=	chromodacryorrhoea
Da	=	distended abdomen
Dh	=	dehydration
E	=	pallor of the extremities
H	=	hunched posture
L	=	lethargy
Oc	=	corneal opacity
P	=	pilo-erection
Pt	=	ptosis
Rd	=	decreased respiratory rate
Rg	=	gasping respiration
Rg*	=	occasional gasping respiration
Ri	=	increased respiratory rate
Rl	=	laboured respiration
Rn	=	noisy respiration
Rn*	=	occasional sneezing
S	=	increased salivation
Sa	=	red/brown staining around ano-genital region
Se	=	red/brown staining around eyes
Sf	=	red/brown staining of fur
Sm	=	red/brown staining around mouth
Ss	=	red/brown staining around snout
Ss*	=	colourless nasal discharge
Wf	=	wet fur
Wt	=	tiptoe gait
0	=	no abnormalities detected
X	=	animal dead
X•	=	animal died immediately after observations performed
X*	=	animal killed *in extremis*
( )	=	additional observations noted prior to killing *in extremis* (animal no 27)

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 6

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 1

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING EXPOSURE (HOUR)		EFFECTS NOTED POST EXPOSURE (HOUR)	EFFECTS NOTED DURING DAY OF OBSERVATION						
		½	1#	1	1	2	3	4	5	6	7
2.38*	1 Male	Rd	WfHPRiPt	HPRdRIPtRn	HPRIRdRg RnSsSm	HPRIRdRnL DaDhESs Sm	HLPRdRIRg DaRnSmCh DhEX*				
	2 Male	0	WfHPRiPt Rn	HPRIRnRn*	HPRIRiRn	HPRIRiRn DhSe	HPRn*RIRn	H	HRd	Rn*	Rn*
	3 Male	0	WfHPRiPt	HPRnPt	HPRnRIRi Se	HPRIRiRn	HPRiRI	H	HRi	HRIRn*	HRn*
	4 Male	Rd	WfHPSe RdPt	HPRdRIRgPtRn Rn*	HPRIRdSe	HPRIRnRd DhSs	HPRdRIRn* DhRn	HP	HRn*	HRn*	Rn*
	5 Male	0	WfHPRiPt	HPRiRIPtRn	HPPtRIRi SeSRg	HPRIRiRg DhRnSeSs	HPRiRnRI	H	HRiRn*	HRn*Rn RI	HRn*RI
	6 Female	0	WfHPRiPt	HPPtRn	HPRIRdRn SsSmE	HERIRdRnP SfWtDhSa	HPDhWt RdRIERn	HP	HRd	0	0
	7 Female	Rd	WfHPRiPt	HPPtRd	HPRIRiRnE	HPRiRnRn*E	HPRiRnRn*	H	HRn*	HRn*	Rn*
	8 Female	Wf	WfHPRiPt	HPPtRn*Rn	HPRIRiSe SfE	HRIRiRnSaP DhSsSmWt	HPRiRIDh WtSa	H	HRISa	HRI	HRI
	9 Female	0	WfHPRdPt	HPRdPt	HPERIRiSe Sf	HPRIRiESe	HPRdRI	H	HRI	H	H
	10 Female	0	WfHPRiPt	HPRdPtRn*Rn	HPRIRiSm	HPRiRn*	HPRiRn*	HP	HRi	H	0

* = one hour exposure

\# = immediately after removal of animals from restraining tube

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 6 (continued)

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 1

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING DAY OF OBSERVATION													
		8	9	10	11	12	13	14	15	16	17	18	19	20	21
2.38*	1 Male	Rn*	RiRI Rn*	RiRn*	Rn*	Rn*	Rn*	Rn*	Rn*	0	0	0	0	0	0
	2 Male	HRn*	H	0	0	0	0	0	0	0	0	0	0	0	0
	3 Male	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	0	Rn*	Rn*	Rn*	Rn*	Rn*
	4 Male	HRn* RI	RIRn*	RdRI	HRdRI SsRn	HRdRI SsRn	HRd Rn*	HRn* Rd	Rn*Rd RI	Rn*Rd	RdRn*	Rn*Rd Se	Rn*	Rn*	Rn*
	5 Male	H	0	0	0	0	0	SsHRI	SsHRI Rn*	RISs	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss
	6 Female	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	Rn*	0	Rn*	Rn*	Rn*	Rn*
	7 Female	HRn* RI	HRn*	HRn*	Rn*	Rn*	Rn*	SeRn*	Rn*Se Ss	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss	Rn*
	8 Female	H	H	0	0	0	0	0	0	0	0	0	0	0	0
	9 Female	0	0	0	Rn*	Rn*	Rn*	Rn*Ss	Rn*RI Ss	Rn*Ss	Ss	SsRI	RISs	Ss	Rn*
	10 Female														

* = one hour exposure

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 7

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 2

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING EXPOSURE (HOUR)					EFFECTS NOTED POST EXPOSURE (HOUR)	EFFECTS NOTED DURING DAY OF OBSERVATION						
		½	1	2	3	4*	1	1	2	3	4	5	6	7
2.32#	11 Male	0	0	0	0	WfHP RnRiRI	HPRiRIRn	HPRIRiRnE SeWfSmSs	HPCcOcSeL RdRISs*SsSm DhSfEX*					
	12 Male	Rd	0	WfRi	Wf	WfHP RnRi	HPRiPtRn	HPRIRiRnE PtL	HPRIRiERnPt Dh	HPRIPtDh E	PtDhE HLPRn	HPDh WtEPtRI	HPPt	HPSs
	13 Male	Rd	0	Wf	WfRi	WfHP RnRiRI	HPRdRIPtRn	HLPRnRIRi ChSeRgSs SmE	HPRIRiRnEPt RgChSfSsSm Ss*OcX*					
	14 Male	0	0	0	Wf	WfHP RnRiRI	HPRdRIPtRn	HPRIRdRg ChES	HRIRdRgRnP SfSeSmE ChSDhSsX*					
	15 Male	0	Wf	Wf	Wf	WfHP RnRiRI	HPRdRIRn	HLPRIRiRnE PtRgSs	X					
	16 Female	0	0	Ri	WfRi	WfHP RnRi	HPRiRn	HPRIRiSfSm	HPRiRIRn DhSfSm	HPRIDh Rn	HPDh	HPDhRI Rn	HPRI RnRn*	HPRI Rn*
	17 Female	0	Wf	Wf	Wf	WfHP RnRd RIPt	HPRdRIPtRn	HLPRIRdRg RnESeChSf SmSs	HLPRIRdRg RnDhChSe SsSs*SmE OcX*					
	18 Female	Rd	WfRd	Wf	Wf	WfHP RnRi	HPRiRIPtRn	HLPRIRdRg ESmSsSfSe	X					
	19 Female	Wf	Wf	Wf	Wf	WfHP RnRiRI	HPRiPtRIRn	HLPRIRiRnE SmSsS	HPRIRiRn DhSmSsE	HRIRiECh SsSaSmDh PRnX*				
	20 Female	0	0	Wf	Wf	WfHP RnRi	HPRIRn	HRIRiRnRg PEWfSmSs	RIRiRnRn*H PDhSmSsE	HPRiRI RnRn*	HPRn	HRnRn*	HPRn	HPRn

= four hour exposure

* = immediately after removal of animals from restraining tube

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

T A B L E 7 (continued)

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 2

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING DAY OF OBSERVATION													
		8	9	10	11	12	13	14	15	16	17	18	19	20	21
2.32#	11 Male														
	12 Male	HPSs Ri	HPRd	HPRn* Rd	HPRn* RdSs	HPRn* RdSs	HPRn* SsRd	HPRn* RdSs	HPRn* SsRd	HPRd SsRn*	HPSs Rd	HPSs RdRn*	HPRd Rn*Ss	HPRd SsRn*	HPRd SsRn*
	13 Male														
	14 Male														
	15 Male														
	16 Female	HRn	H	0	0	0	0	0	H	0	0	0	0	0	0
	17 Female														
	18 Female														
	19 Female														
	20 Female	HRISs	HRI	HRI	HRn* Ss	HSs Rn*	Rn*Ss	RIRn* Ss	RIRn* Ss	Rn*Ss HRI	HRISs Rn*	HRISs Rn*	,HSs Rn*	HSs Rn*	RIRn* SsH

= four hour exposure

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 8

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 4

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING EXPOSURE (HOUR)				EFFECTS NOTED POST EXPOSURE (HOUR)	EFFECTS NOTED DURING DAY OF OBSERVATION				
		1	2	3	4*	1	1	2	3	4	5
1.76#	31 Male	0	Wf	Wf	WfHPRd RlRn	HPRdRlRn	HPRdRlSSe RnPtE	HLPRdRlRg RnSeDhSm EPtDaS	X		
	32 Male	0	0	Wf	WfHPRi Rn	HPRdRnPt	HPSeRdRl RgRnPt	HPRdRlRg RnSs	HPRdRlRn	HPRdRlRn DhE	HLPRdRlRn DhE
	33 Male	0	WfRi	WfRi	WfHPRd RlRn	HPRdRlPt	HPSsRdRl RgRnSm	HPRdRlRg RnSsSeEDa	HLPRdRlRg RnEDaPtDh Sm	LPRdRlRgH RnESaPtDh SsSmDa	HLPRdRlRg RnPtEDaDh SsSa
	34 Male	0	0	0	WfHPRd RlRn	HPRdRlRnPt	HPSeRlRg RdRnPt	HLPRdRlRg RnSeSsPtS SmDaDh	HLPRdRlRg RnEDaPtSe SmSDh	X	
	35 Male	0	0	Wf	WfHPRd Rn	HPRdRlRn	HPSeRdRl RgRn	HLPRdRlRg RnSeSsSDh DaPtE	HLPRdRlRg RnEDaPtSm DhOcX*		
	36 Female	0	0	0	WfHPRi Rn	HPRdPtRn	HPRdRlSRn	HLPRdRlSs	HLPRdRlERg RnADhPtSm SsDaX*		
	37 Female	0	Wf	Wf	WfHPRd Rn	HPRdPtRn	HLPRdRlRg RnSsS	HLPRdRlRg RnSeSsDa DhESm	X		
	38 Female	Wf	Wf	WfRi	WfHPRd RlESsARn	HLPRdRlAERn Pt	HLPARdRl RgRnSDa	X			
	39 Female	0	0	Wf	WfHPRd Rn	HPPtRnRd	HLPRdRlRg RnSeSSs	HLPRdRlRg RnSeSsDaE DhSm	X		
	40 Female	0	Wf	Wf	WfHPRi Rn	HPPtRn	HPRdRn	HPRdRlPt	HLPRdRlDh Rn	HLPRdRl DhRn	HLPRdRn

= four hour exposure

* = immediately after removal of animals from restraining tube

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

T A B L E 8 (continued)

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 4

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING DAY OF OBSERVATION								
		6	7	8	9	10	11	12	13	14
1.76#	31 Male									
	32 Male	HLPRdRI RnDhE	HLPRdRI RnE	HLPRdRI	HPRdRISs	HPRdRIRn Ss	Rn*HSs	Rn*HSs	HRISsRn*	HSs
	33 Male	HLPRdRI RgRnPtDh DaSsSaE	HLPRdRI RnPtEDh	HLPRdRI Dh	HLPRdRI Ss	HPRn*Rd SsRI	Rn*HRdSs	Rn*HRdSs	HRIRdSs	HRdSs
	34 Male									
	35 Male									
	36 Female									
	37 Female									
	38 Female									
	39 Female									
	40 Female	HLPRdRn	HPRdRn	HPRnRd	HPSsRd	HPSsRd	Rn*HRdSs	Rn*HRdSs	HRdRn*Ss	HSsRd

= four hour exposure

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 9

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 3

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING EXPOSURE (HOUR)				EFFECTS NOTED POST EXPOSURE (HOUR)	EFFECTS NOTED DURING DAY OF OBSERVATION						
		1	2	3	4*	1	1	2	3	4	5	6	7
0.89#	21 Male	0	0	0	WfHP RnSe	HPRnRn*	HPRISs	HRIRn	HPRIRn	HRI	HRnRn*	HSs	Rn*SsH
	22 Male	0	Wf	Wf	WfHPPt Ri	HPRi	HPRdRI Rn*	HPRIRd	HPRdRI RnPtSs	HPtRnRI Rd	HPRnRI Rn*	HPRi	Rn*SsH
	23 Male	Wf	Wf	Wf	WfHPRi	HPRi	HP	HRn*Se Ss	HRnSe SsRd	RnSeSsH	HSs	HRd	HRISs
	24 Male	0	Wf	WfRi	WfHP RnRi	HPRn	HPRdRI Rn*	HPRdRI SsPtRn*	HPRdRI RnSsPt	HPRd	HPRI	HPRISs	HPRISs
	25 Male	0	0	0	WfHPSs Ri	HPRi	HPRi	H	HRdSs	HRd	H	H	0
	26 Female	0	Wf	Wf	WfHP Rn	HP	HP	H	H	H	H	0	0
	27 Female	0	0	0	WfHP RnRi	HPRiRn	HPSeRd RISm	PRdRIRg HSsSeRn PtEX*(A)					
	28 Female	Wf	Wf	Wf	WfHPRi	HPRi	HPRn*	HPSeSs	HSsSe	HSe	HSe	H	HSe
	29 Female	0	0	0	WfHP RnRi	HPRiRn	HPRdRI Rn*	HPRn*	HSsRn*	HWtRdRI	HPSeSs Rd	HPSeRd RI	HPSeRdRI Rn*Ss
	30 Female	Wf	Wf	WfRi	WfHPRi	HPRi	HPRn*	Rn*	HSeSs Rn*	HWtRgRd RISeRn*	HSeSsRd RIWtRn	HRdRI Rg*RnSs	HRn*Rg* RnRdRISs

= four hour exposure

* = immediately after removal of animals from restraining tube

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

T A B L E 9 (continued)

INDIVIDUAL CLINICAL OBSERVATIONS - DOSE GROUP 3

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	EFFECTS NOTED DURING DAY OF OBSERVATION						
		8	9	10	11	12	13	14
0.89#	21 Male	Rn*Ss	Rn*SsRl	Rn*Rl	Rn*Rl	Rn*Ss	Rn*Ss	Rn*Ss
	22 Male	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss	Rn*Ss	0
	23 Male	HRdRlSs	HRdRlSs	RdSs	RdRn*Ss	RdSs	RdRn*Ss	Rn*Ss
	24 Male	HSs	HSs	Ss	Ss	Ss	Ss	Ss
	25 Male	0	0	0	0	0	0	0
	26 Female	0	0	0	0	0	0	0
	27 Female							
	28 Female	H	H	0	0	0	0	0
	29 Female	SeHPSsRl	SeHPSsRn*Rl Rg	HPSsRnRl	HPSsRn	HSsRnRn*	HRn*Ss	HSsRd
	30 Female	RnSsRlRn*	Rn*SsRl	Rn*Ss	Rn*Ss	Rn*Ss	Ss	Ss

= four hour exposure

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 10

INDIVIDUAL BODYWEIGHTS - DOSE GROUP 1

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	BODYWEIGHT (g) ON DAY:					INCREMENT (g) DURING WEEK:		
		0	7	14	21	AT DEATH	1	2	3
	1 Male	231	-	-	-	169	-	-	-
	2 Male	236	222	276	324		-14	54	48
	3 Male	247	261	326	366		14	65	40
	4 Male	243	244	286	339		1	42	53
2.38*	5 Male	232	221	262	274		-11	41	12
	6 Female	227	215	229	252		-12	14	23
	7 Female	223	233	259	272		10	26	13
	8 Female	236	198	226	254		-38	28	28
	9 Female	232	216	236	255		-16	20	19
	10 Female	232	216	228	246		-16	12	18

* = one hour exposure

- = animal dead

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 11

INDIVIDUAL BODYWEIGHTS - DOSE GROUP 2

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	BODYWEIGHT (g) ON DAY:					INCREMENT (g) DURING WEEK:		
		0	7	14	21	AT DEATH	1	2	3
2.32#	11 Male	244	-	-	-	193	-	-	-
	12 Male	234	207	255	295		-27	48	40
	13 Male	241	-	-	-	180	-	-	-
	14 Male	267	-	-	-	193	-	-	-
	15 Male	237	-	-	-	183	-	-	-
	16 Female	225	208	231	254		-17	23	23
	17 Female	236	-	-	-	186	-	-	-
	18 Female	235	-	-	-	195	-	-	-
	19 Female	230	-	-	-	165	-	-	-
	20 Female	221	214	217	235		-7	3	18

= four hour exposure

- = animal dead

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 12

INDIVIDUAL BODYWEIGHTS - DOSE GROUP 4

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	BODYWEIGHT (g) ON DAY:				INCREMENT (g) DURING WEEK:	
		0	7	14	AT DEATH	1	2
1.76#	31 Male	298	-	-	212	-	-
	32 Male	304	234	277		-70	43
	33 Male	297	203	254		-94	51
	34 Male	300	-	-	199	-	-
	35 Male	283	-	-	200	-	-
	36 Female	227	-	-	172	-	-
	37 Female	201	-	-	157	-	-
	38 Female	203	-	-	174	-	-
	39 Female	211	-	-	162	-	-
	40 Female	211	188	208		-23	20

= four hour exposure

- = animal dead

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 13

INDIVIDUAL BODYWEIGHTS - DOSE GROUP 3

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	ANIMAL NUMBER AND SEX	BODYWEIGHT (g) ON DAY:				INCREMENT (g) DURING WEEK:	
		0	7	14	AT DEATH	1	2
0.89#	21 Male	272	243	284		-29	41
	22 Male	310	285	312		-25	27
	23 Male	292	304	354		12	50
	24 Male	274	221	267		-53	46
	25 Male	308	353	390		45	37
	26 Female	261	271	282		10	11
	27 Female	232	-	-	178	-	-
	28 Female	247	247	274		0	27
	29 Female	251	237	242		-14	5
	30 Female	263	257	278		-6	21

= four hour exposure

- = animal dead

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 14

INDIVIDUAL NECROPSY FINDINGS - DOSE GROUP 1

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	MACROSCOPIC OBSERVATION	ANIMAL NUMBER AND SEX									
		1■ M	2 M	3 M	4 M	5 M	6 F	7 F	8 F	9 F	10 F
	Lungs: swollen	P									
	abnormally red	P									
	dark patches			P							P
	Liver: right lobe enlarged and hardened										P
2.38*	Spleen: pale	P									
	Kidneys: pale	P									
	Small and Large Intestines: congestion	P									
	gaseous distension	P									
	No abnormalities detected (N)		N		N	N	N	N	N	N	

M = male F = female * = one hour exposure ■ = animal killed *in extremis* P = finding present

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 15

INDIVIDUAL NECROPSY FINDINGS - DOSE GROUP 2

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	MACROSCOPIC OBSERVATION	ANIMAL NUMBER AND SEX									
		11▲ M	12 M	13■ M	14■ M	15▲ M	16 F	17■ F	18▲ F	19■ F	20 F
	Lungs: swollen			P	P				P		
	abnormally red	P				P		P	P	P	
	pale			P	P						
	dark patches	P		P		P			P	P	
	Liver: pale							P			
	patchy pallor	P				P			P		
2.32#	Spleen: pale	P		P					P		
	Small intestine: congestion	P		P	P	P		P	P	P	
	gaseous distension	P		P	P	P		P	P	P	
	Large intestine: congestion					P			P		
	gaseous distension					P			P		
	No abnormalities detected (N)		N				N				N

M = male F = female # = four hour exposure ▲ = animal died during study ■ = animal killed *in extremis* P = finding present

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 16

INDIVIDUAL NECROPSY FINDINGS - DOSE GROUP 4

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	MACROSCOPIC OBSERVATION	ANIMAL NUMBER AND SEX									
		31▲ M	32 M	33 M	34▲ M	35■ M	36■ F	37▲ F	38▲ F	39▲ F	40 F
	Lungs: swollen					P		P	P		
	abnormally red				P						
	pale					P		P		P	
	dark patches	P			P		P	P	P	P	
	Liver: dark				P				P		
	patchy pallor	P					P	P		P	
1.76#	Spleen: small				P		P	P		P	
	pale	P									
	Kidneys: dark				P						
	Stomach: gaseous distension				P						
	Small intestine: congestion	P					P	P	P	P	
	gaseous distension	P			P	P	P	P	P	P	
	reddened				P						
	Large intestine: gaseous distension				P	P	P		P	P	
	reddened				P						
	No abnormalities detected (N)		N	N							N

M = male F = female # = four hour exposure ▲ = animal died during study ■ = animal killed *in extremis* P = finding present

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

TABLE 17

INDIVIDUAL NECROPSY FINDINGS - DOSE GROUP 3

MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	MACROSCOPIC OBSERVATION	ANIMAL NUMBER AND SEX									
		21 M	22 M	23 M	24 M	25 M	26 F	27■ F	28 F	29 F	30 F
	Lungs: abnormally red							P			
0.89#	dark patches							P			
	Small and Large Intestines: gaseous distension							P			
	No abnormalities detected (N)	N	N	N	N	N	N		N	N	N

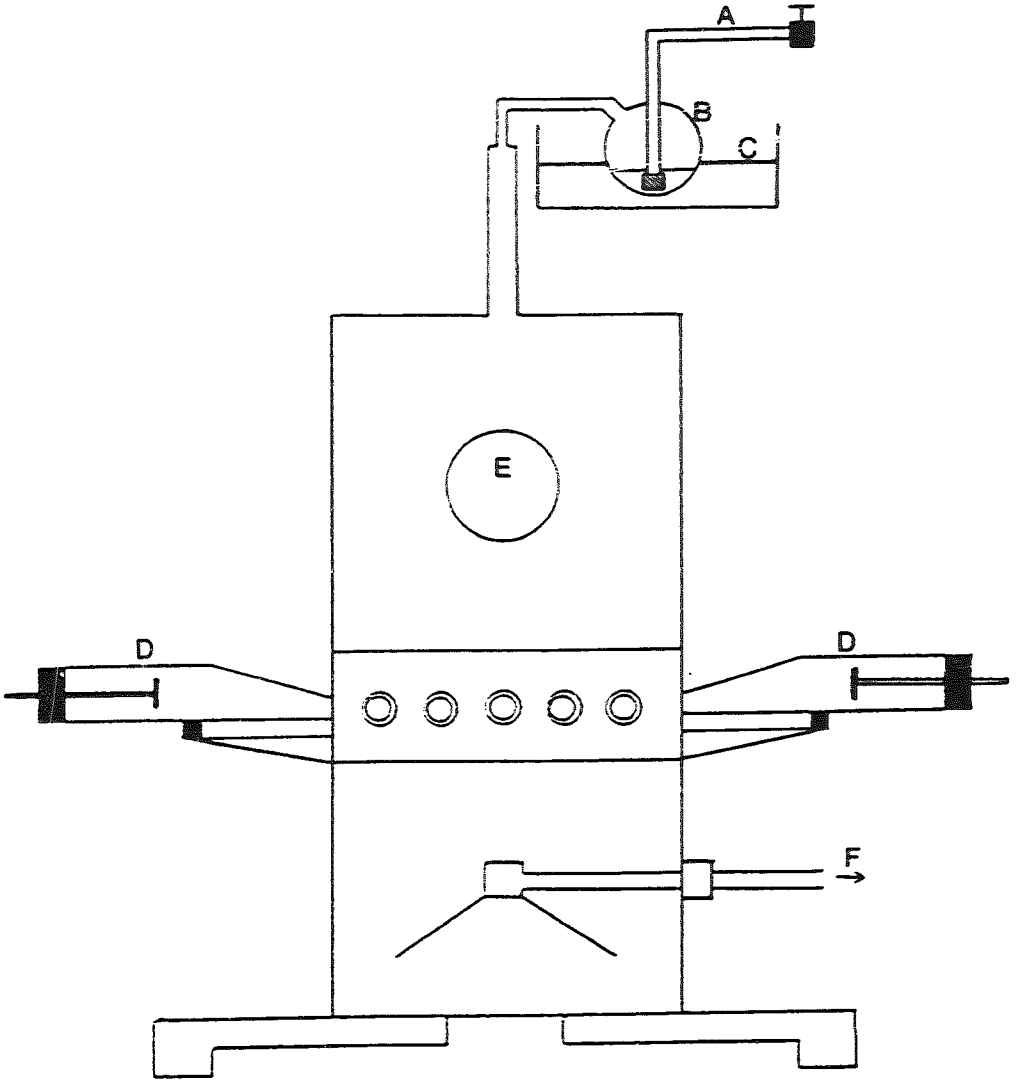
M = male F = female # = four hour exposure ■ = animal killed *in extremis* P = finding present

FIGURE

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

FIGURE I

SCHEMATIC DIAGRAM OF THE DYNAMIC SYSTEM USED FOR NOSE ONLY EXPOSURE OF RATS



A = Metered air supply	D = Animal restraining tube
B = Glass flask	E = Observation port
C = Heated water bath	F = Metered vacuum exhaust

APPENDICES

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

APPENDIX I

EXPOSURE CHAMBER TEMPERATURE

TIME (MINUTES)	CHAMBER TEMPERATURE (°C) DURING EXPOSURE		
	GROUPS 1 + 2 (2.38 mg/l + 2.32 mg/l)	GROUP 4 (1.76 mg/l)	GROUP 3 (0.89 mg/l)
0	20	20	21
30	21	20	21
60	20	19	21
90	20	20	21
120	20	19	21
150	20	19	21
180	20	19	21
210	19	20	21
240	19	20	21

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

APPENDIX II

EXPOSURE CHAMBER RELATIVE HUMIDITY

TIME (MINUTES)	RELATIVE HUMIDITY (%) DURING EXPOSURE		
	GROUPS 1 + 2 (2.38 mg/l + 2.32 mg/l)	GROUP 4 (1.76 mg/l)	GROUP 3 (0.89 mg/l)
0	54	47	65
30	62	47	59
60	64	51	62
90	65	56	63
120	67	59	67
150	69	59	66
180	68	57	64
210	72	54	69
240	69	53	66

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

APPENDIX III

EXPOSURE CHAMBER OXYGEN CONCENTRATION

TIME (MINUTES)	OXYGEN CONCENTRATION (%) DURING EXPOSURE		
	GROUPS 1 + 2 (2.38 mg/l + 2.32 mg/ll)	GROUP 4 (1.76 mg/l)	GROUP 3 (0.89 mg/l)
0	20.0	20.5	20.5
30	19.6	-	-
60	20.1	-	-
90	-	-	-
120	20.2	20.5	20.4
150	-	-	-
180	-	-	-
210	-	-	-
240	20.2	20.5	20.4

- = not determined

2-ACETYLFURAN : ACUTE INHALATION TOXICITY (NOSE ONLY) STUDY IN THE RAT

APPENDIX IV

TEMPERATURE AND RELATIVE HUMIDITY IN TEST ROOM

GROUP NUMBER	MEAN ACHIEVED ATMOSPHERE CONCENTRATION (mg/l)	TEMPERATURE (°C)		RELATIVE HUMIDITY (%)
		MAXIMUM	MINIMUM	
1 + 2	2.38 + 2.32	20	19	48 - 52
4	1.76	20	19	48 - 52
3	0.89	21	19	48 - 53

SPL PROJECT NUMBER: 541/019

APPENDIX V

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE
IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY	TEST TYPE
SafePharm Laboratories Ltd. P.O. Box No. 45 Derby DE1 2BT	Analytical Chemistry Environmental Tox. Environmental Fate Mutagenicity Phys/Chem. tests Toxicology

DATE OF INSPECTION

22 January 1996

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of non-clinical studies performed at these facilities.

D.F. Moore

27/2/96

D F Moore
Director
UK GLP Monitoring Authority

APPENDIX VI

CHEMICAL ANALYSIS OF CHAMBER ATMOSPHERES

1. INTRODUCTION

A gas chromatographic (GC) method of analysis was developed and used to measure the chamber atmosphere concentrations of 2-ACETYLFURAN in an acute inhalation study.

The method was validated with respect to linearity, specificity and accuracy prior to use in the study.

2. METHOD OF ANALYSIS

Preliminary investigations showed that when test atmosphere containing 2-ACETYLFURAN was passed through a series of acetonitrile impingers, no significant levels were collected in the second and subsequent impingers.

During the study each sample consisted of three litres of test atmosphere drawn through one impinger containing acetonitrile (50 ml). The 2-ACETYLFURAN concentration in the impingers was determined by gas chromatography (GC) using an external standard technique.

2.1 Samples

The test impinger samples were analysed without further dilution.

2.2 Standards

Aliquots (~0.1g) of test material were accurately weighed and quantitatively diluted with acetonitrile to give a concentration of 0.06, 0.08 or 0.3 mg/l.

2.3 Procedure

The standard and sample solutions were analysed by GC using the following conditions:

Column	:	DB-Wax (30 m x 0.32 mm id x 0.25 μm film)
Oven temperature program	:	initial - 50°C rate - 10°C/min final - 150°C
Injection temperature	:	150°C
Flame ionization detector temperature	:	200°C
Injection volume	:	1 μl

3. CALCULATIONS

The amount of test material collected in the impingers was calculated using Equation 1.

$$TM = \frac{A_{spl} \times W_{std} \times D_{spl} \times 10^3}{A_{std} \times D_{std}} \qquad \text{Equation 1}$$

where:

- TM = amount of test material collected in impinger (mg)
- A_{spl} = mean peak area for sample solution
- A_{std} = mean peak area for standard solution, corrected to nominal standard concentration
- W_{std} = weight of standard material taken (g)
- D_{std} = dilution factor for standard solution
- D_{spl} = dilution factor for sample solution

The concentration of test material in the atmosphere was calculated using Equation 2.

$$C_{atm} = \frac{TM}{V} \qquad \text{Equation 2}$$

- C_{atm} = concentration of test material in test atmospheres (mg/l)
- V = volume of test atmosphere sampled through impinger (l)

DOSE GROUPS 1 AND 2

SAMPLE NUMBER	START OF SAMPLING DURING EXPOSURE (minutes)	VOLUME OF ATMOSPHERE SAMPLED THROUGH IMPINGER (l)	AMOUNT FOUND IN IMPINGER (mg)	ATMOSPHERE CONCENTRATION (mg/l)
22	0	3	ND	-
23	28	3	8.07	2.69
24	55	3	6.2	2.07
25	115	3	6.64	2.21
27	175	3	8.26	2.76
28	235	3	5.65	1.89

ND = none detected - = not applicable

DOSE GROUP 4

SAMPLE NUMBER	START OF SAMPLING DURING EXPOSURE (minutes)	VOLUME OF ATMOSPHERE SAMPLED THROUGH IMPINGER (l)	AMOUNT FOUND IN IMPINGER (mg)	ATMOSPHERE CONCENTRATION (mg/l)
61	0	3	ND	-
62	15	3	5.34	1.78
63	55	3	4.69	1.56
64	116	3	4.92	1.64
66	176	3	5.60	1.87
67	235	3	5.81	1.94

ND = none detected - = not applicable

DOSE GROUP 3

SAMPLE NUMBER	START OF SAMPLING DURING EXPOSURE (minutes)	VOLUME OF ATMOSPHERE SAMPLED THROUGH IMPINGER (l)	AMOUNT FOUND IN IMPINGER (mg)	ATMOSPHERE CONCENTRATION (mg/l)
36	0	3	ND	-
37	20	3	2.85	0.949
38	55	3	2.85	0.948
39	118	3	2.65	0.884
41	175	3	2.68	0.892
42	238	3	2.28	0.759

ND = none detected - = not applicable

4. METHOD VALIDATION

4.1 Linearity

A range of standard solutions was prepared covering the concentration range 0 to 1.0 mg/l, and analysed.

The detector response was shown to be linear up to 1 mg/l.

Standard concentration (mg/ml)	Peak area (units)
0	0
0.01	1.723×10^3
0.04	6.735×10^3
0.06	1.098×10^4
0.08	1.444×10^4
0.16	2.969×10^4
Slope	1.862×10^5
Intercept	-2.643×10^2
Correlation coefficient	1.000

Standard concentration (mg/ml)	Peak area (units)
0	0
0.25	8.310×10^4
0.40	1.261×10^5
0.50	1.606×10^5
0.60	2.004×10^5
1.00	3.273×10^5
Slope	3.281×10^5
Intercept	-7.961×10^2
Correlation coefficient	1.000

The results are presented graphically on pages 61 and 62.

4.2 Specificity

The diluent solvent (acetonitrile) and a blank impinger (control) were analysed.

Sample	Concentration found
Acetonitrile	None detected
Blank impinger (control)	None detected

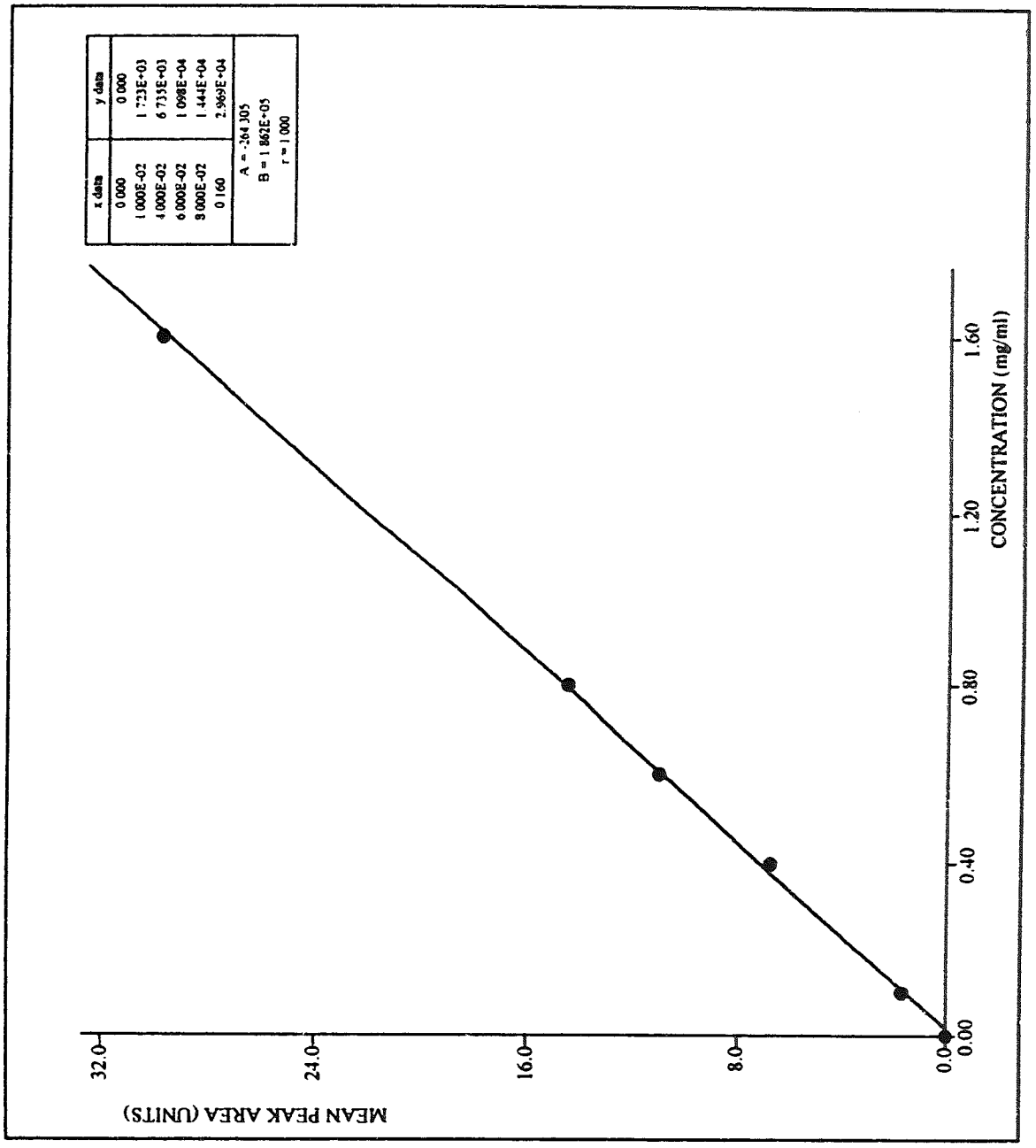
4.3 Accuracy

Blank impingers were accurately fortified with known amounts of test material, three litres of air drawn through and analysed.

Fortification (mg/ml)	Concentration found (mg/ml)	% fortification recovered	Mean recovery (%)
0.0943	0.0876	93	98
0.0831	0.0861	104	

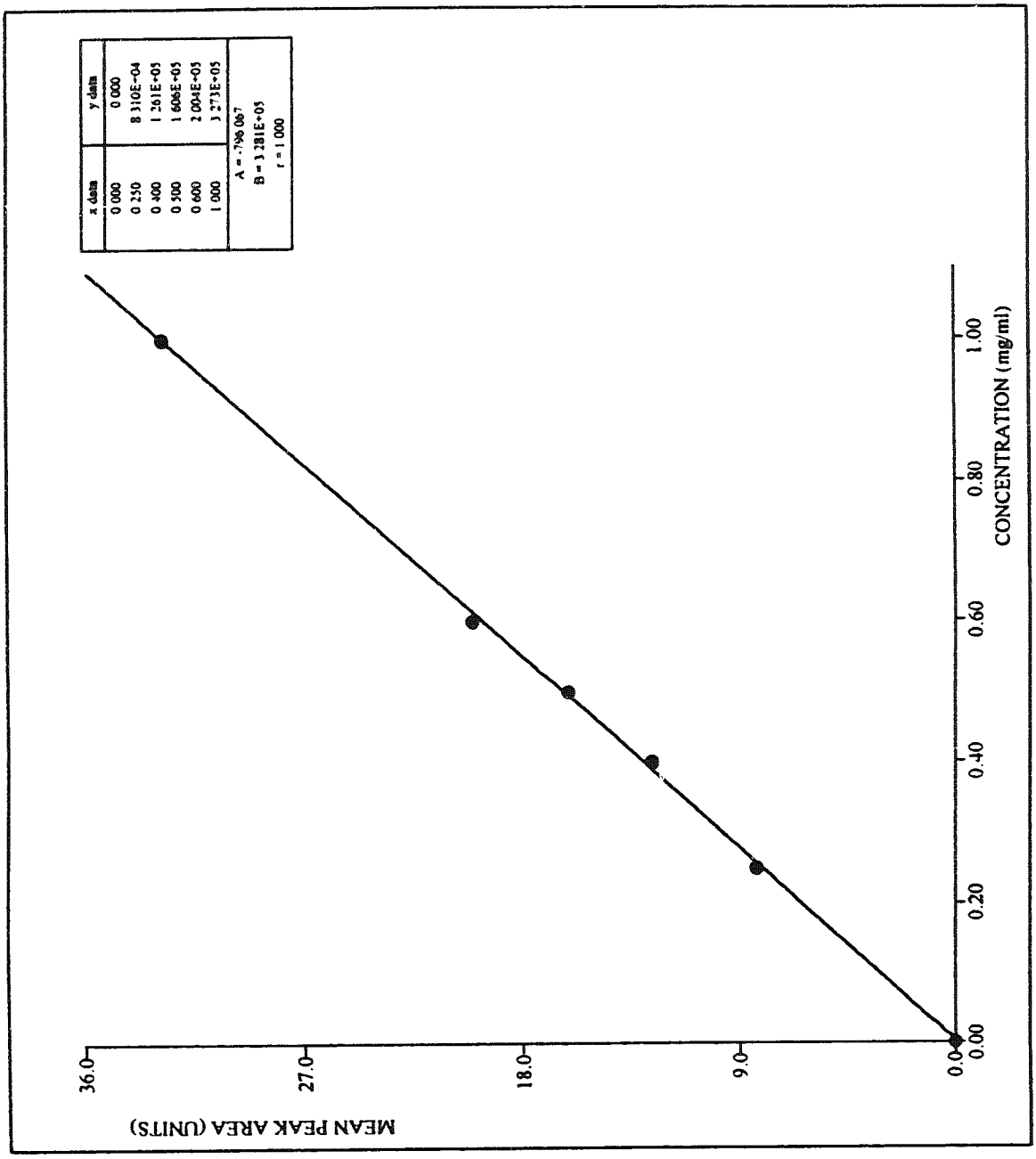
SPL PROJECT NUMBER: 541/019

LINEARITY OF DETECTOR RESPONSE



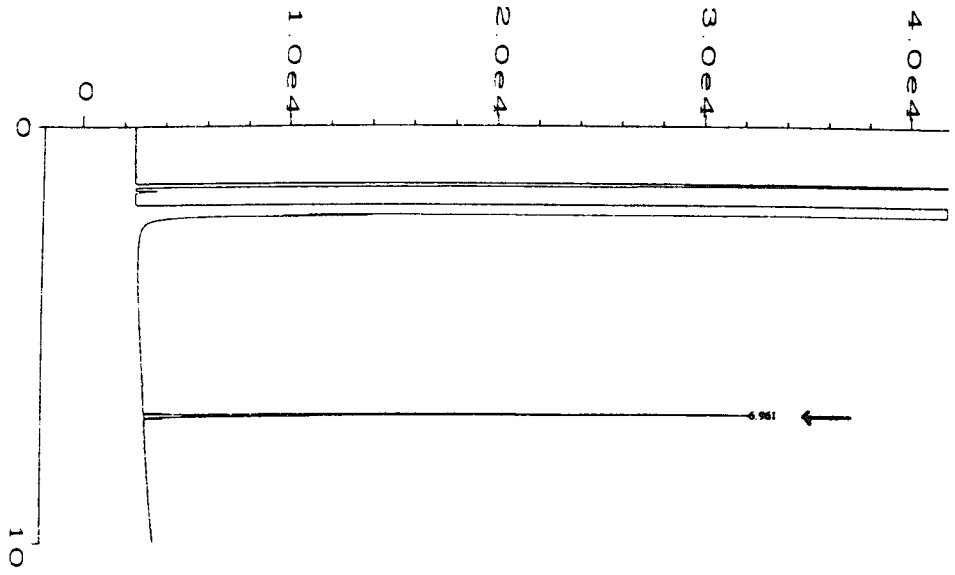
SPL PROJECT NUMBER: 541/019

LINEARITY OF DETECTOR RESPONSE

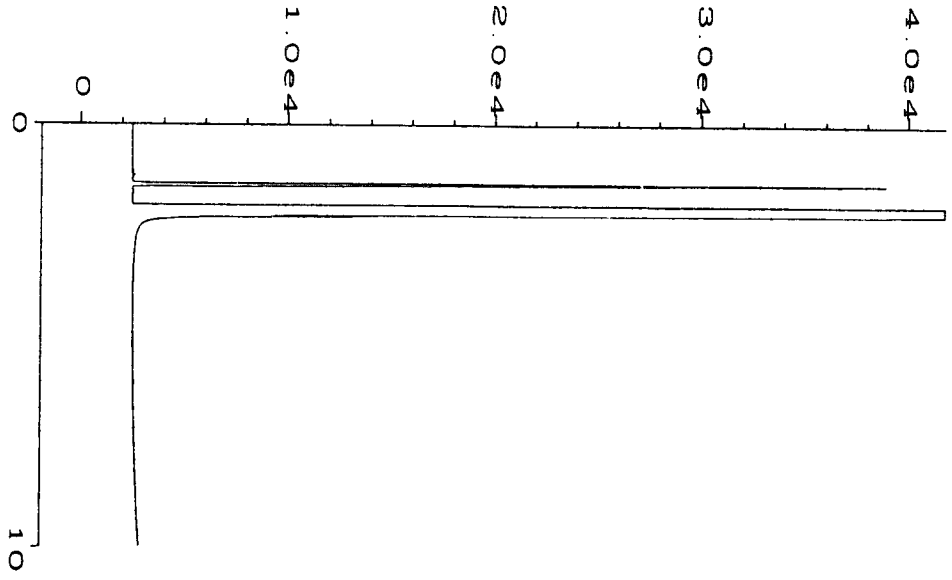


Examples of the typical chromatography generated during this study are given below:

0.3 mg/ml Standard Solution

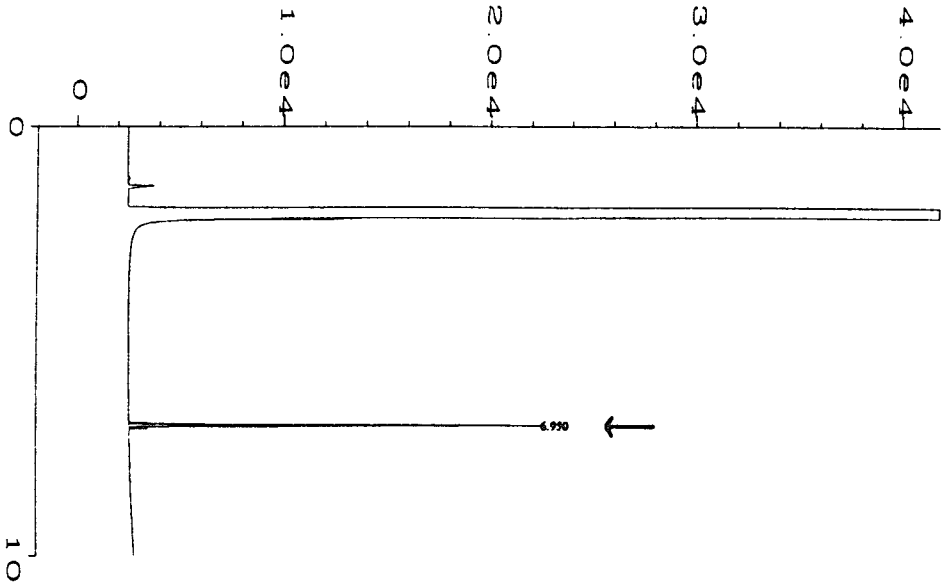


Sample No. 22 - Control Impinger - Dose Groups 1 and 2



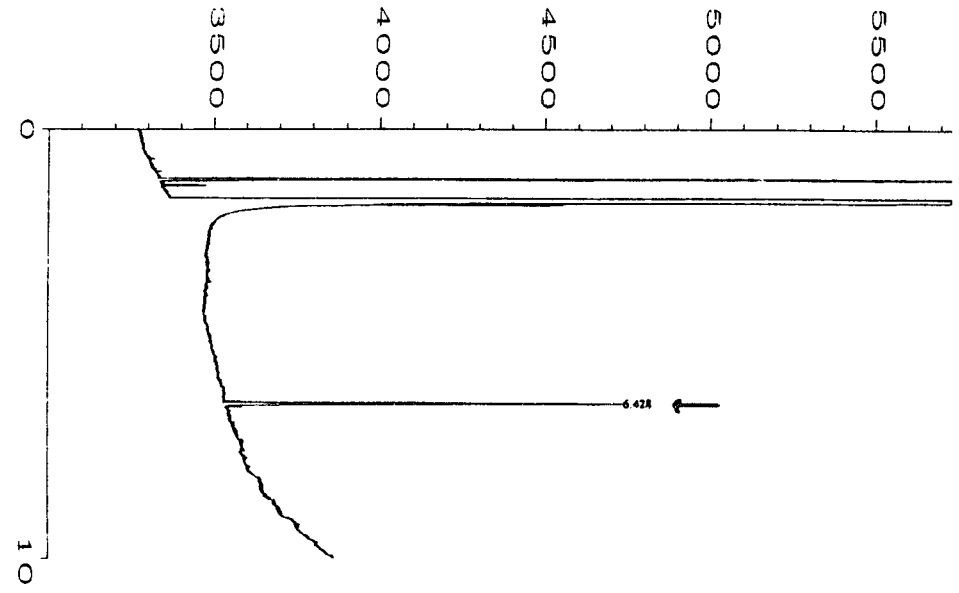
Examples of the typical chromatography generated during this study are given below:

Sample No. 23 - Impinger Sample - Dose Groups 1 and 2

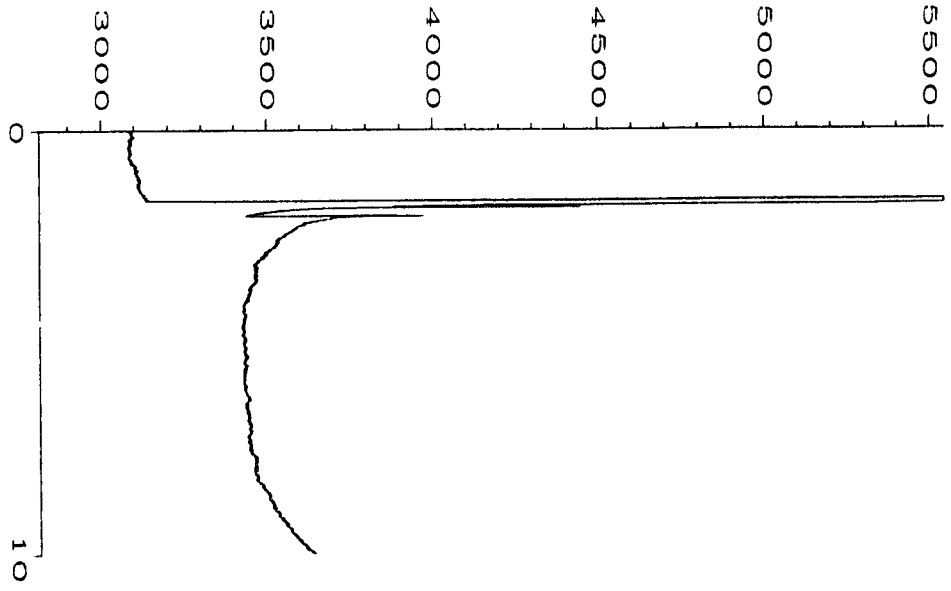


Examples of the typical chromatography generated during this study are given below:

0.08 mg/ml Standard Solution

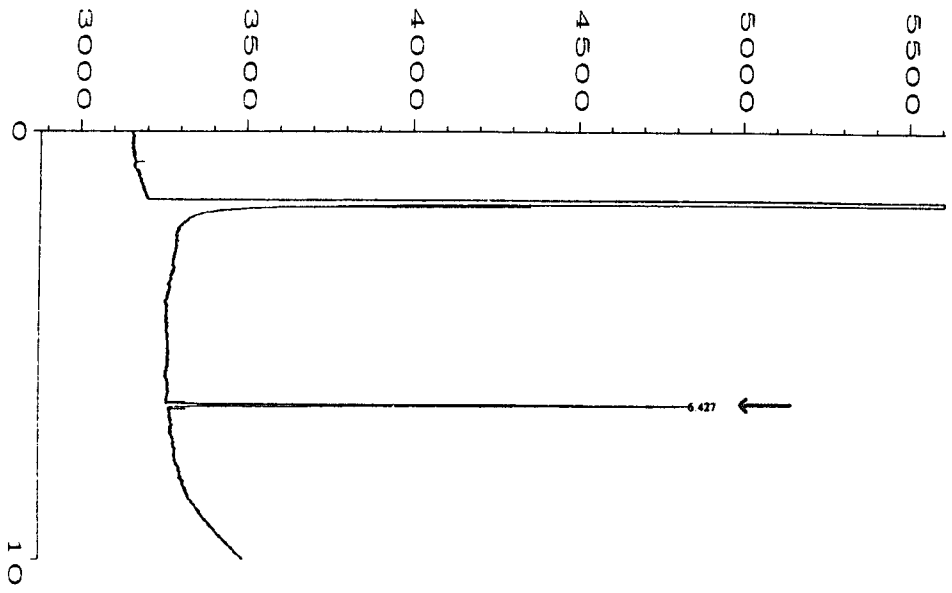


Sample No. 61 - Control Impinger - Dose Group 4



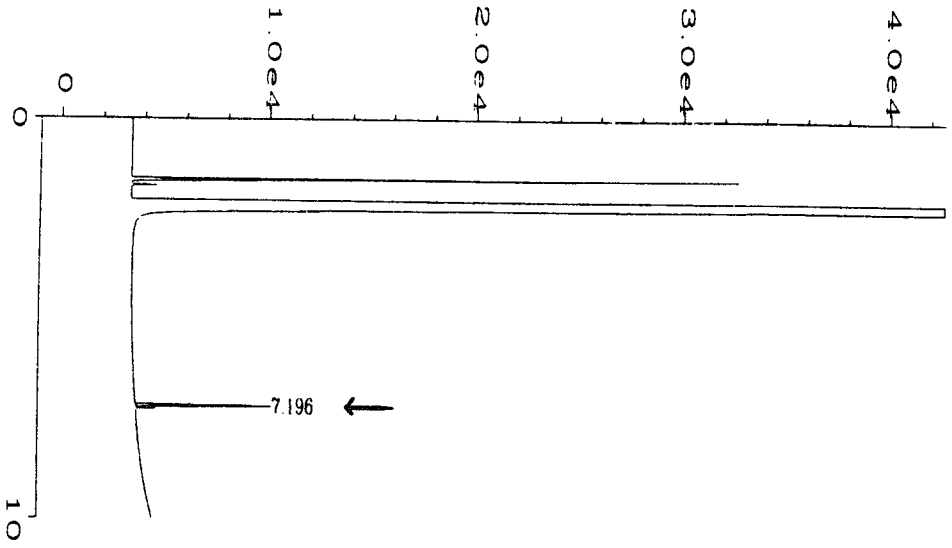
Examples of the typical chromatography generated during this study are given below:

Sample No. 62 - Impinger Sample - Dose Group 4

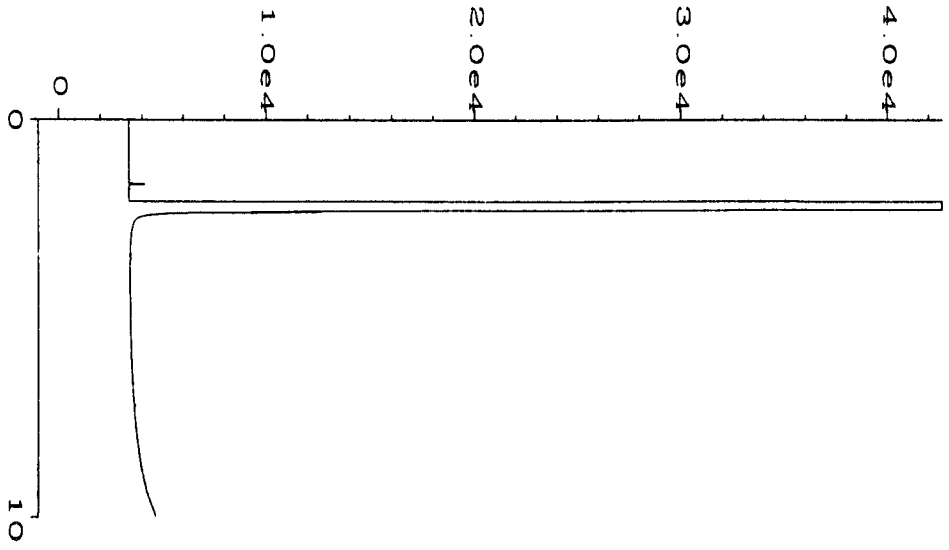


Examples of the typical chromatography generated during this study are given below:

0.06 mg/ml Standard Solution

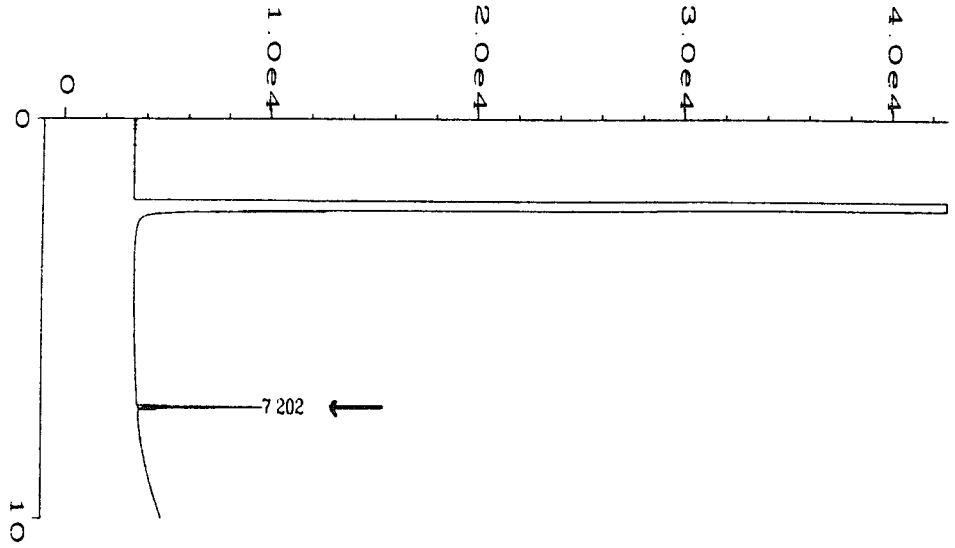


Sample No. 36 - Control Impinger Sample - Dose Group 3



Examples of the typical chromatography generated during this study are given below:

Sample No. 37 - Impinger Sample - Dose Group 3



APPENDIX VII

QO Chemicals, Inc.

INDUSTRIEPARK – B-2440 GEEL (Belgium)
Tel. (014) 58.95.72 – (014) 58.96.91
Fax (014) 58.08.96 – Telex 34827

Great Lakes Fine Chemicals
T. Jones
Lower Road
Halebank
WIDNES, CHESHIRE WA8 8NS
GROOT-BRITTANIE

July 11, 1996

CERTIFICATE OF ANALYSIS

2-ACETYLFURAN

Lot N° : 95E16
Quantity : 1 kg
Sample N° : Q1859
Ref. N° : W0171

Analysis

2-Acetylfuran assay %:	99.7
2,5-Diacetylfuran area %:	not detected
Water wt %.	0.03

J. Maes
supervisor analytical services

cc : W. Van Rhijn
G.C. Lalande
L. Clark
M. Raeymaekers

JM/mh-qc-acfl107

SPL PROJECT NUMBER: 541/019

APPENDIX VIII

SafePharm Laboratories
P.O. Box 45 Derby DE1 2BT. England
Tel: (01332) 792896 Fax: (01332) 799018

PROTOCOL

PROJECT NO: 0541/019

TEST MATERIAL	:	2-ACETYLFURAN
STUDY TYPE	:	Acute Inhalation Toxicity (Nose Only) in the Rat
PROPOSED START DATE	:	August 1996
PROPOSED COMPLETION DATE	:	September 1996
TARGET (DRAFT) REPORT DATE	:	October 1996
SPONSOR	:	Great Lakes Fine Chemicals Limited Halebank WIDNES Cheshire WA8 8NS

AUTHORISED BY: ..... DATE : 31 JUL 1996
S M Blagden FIAT
STUDY DIRECTOR

AUTHORISED FOR SPONSOR BY: ..... DATE : 01/08/96

APPENDIX VIII (continued)

ACUTE INHALATION (NOSE ONLY) TOXICITY STUDY IN THE RAT

1. **INTRODUCTION AND OBJECTIVES**

To assess the acute inhalation one and (four-hour exposure) toxicity of a test material in the rat by nose-only exposure. The study is designed to comply with OECD Guidelines for Testing of Chemicals 1981 No. 403 "Acute Inhalation Toxicity" referenced as Method B2 in Commission Directive 92/69/EEC "Acute Toxicity-Inhalation" (which constitutes Annex V of Council Directive 67/548/EEC) and to provide information suitable for classification according to international transport regulations. The results of the study are believed to be of value in predicting the likely toxicity in man by the inhalation route.

The work will be performed in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom Compliance Programme, Department of Health, 1989). These Principles are in accordance with GLP standards published as OECD Environment Monograph No 45 (OECD/GD(92)32); and are in conformity with, and implement, the requirements of Directives 87/18/EEC and 88/320/EEC.

2. **ANIMALS**

Specification: Male and female Sprague-Dawley CD strain rats obtained from Charles River (UK) Limited, Margate, Kent. Young adult animals will be used within the weight range 180-350g. Weight variation will not exceed ± 20% of the mean weight for either sex.

Justification: Preferred species of choice as historically used for safety evaluation studies and specified by appropriate regulatory authorities.

3. **ANIMAL HUSBANDRY**

Environment:
- Temperature: 19 - 25°C
- Humidity: 30 - 70%
- Lighting: Twelve hours of artificial light in each twenty-four hour period.
- Ventilation: At least fifteen air changes per hour.

Housing: Groups of up to five by sex in solid-bottomed polypropylene cages with stainless steel mesh lids furnished with softwood flakes (Datesand Ltd., Cheshire, UK).

Diet and Water: Rat and Mouse SQC Expanded Diet No. 1 (Special Diets Services Limited, Witham, Essex, UK), and tap water *ad libitum*.

APPENDIX VIII (continued)

The diet, drinking water and bedding are considered not to contain any contaminants that could reasonably be expected to affect the purpose or integrity of the study

4. **PRE-TEST PROCEDURES**

Acclimatisation Period: At least five days.

Allocation: Animals will be allocated to dose groups using total randomisation procedure

Identification: Each animal, selected at random, will be uniquely identified within the study by ear-punch. A colour-coded cage card will be prepared with details of test material, project number, dose level, sex, numbers of animals, route of administration and Study Director responsible for the study.

5. **TEST MATERIAL**

Identification: Supplied by Study Sponsor with details of purity and hazardous properties if known.

Storage: Room temperature unless otherwise specified by Sponsor

6. **PROCEDURE**

Justification: Inhalation route selected as a possible route of human exposure.

Main Study: A group of twenty animals (ten males and ten females) will be exposed to a vapour atmospheric of the test material. Half the animals (five males and five females) will be exposed for one hour and the others will be exposed for four hours. Based on the results of a pre-study sighting, a maximum concentration of up to 20 mg/l will be used. If exposure to 20 mg/l or to a maximum acceptable concentration results in no mortalities then no further exposures will be undertaken. The maximum acceptable concentration may be limited by humane considerations.

If a significant number of mortalities occur, (ie, two or more per sex), or if the initial concentration is limited by anticipated toxicity then two further groups each of twenty animals (ten males and ten females) will be similarly exposed to concentrations selected to achieve a range of toxic effects and mortality rates. If possible the data should permit an acceptable determination of the LC_{50} for each exposure period. If this is not possible a 4 hour LC_{50} value will be calculated and an estimate of the 1 hour LC_{50} will be made.

APPENDIX VIII (continued)

Animal Exposure Conditions: Animals held in restraining tubes and exposed "nose only" for a single continuous one or four hour period under dynamic air flow conditions in a 30 litre cylindrical exposure chamber. (ADC Developments Ltd., Hitchin, Herts, UK).

Test Atmosphere Generation: An appropriate system will be developed for generating a suitable vapour atmosphere from the test material. If necessary, and after consultation with the Study Sponsor, the test material may be heated to produce the required concentration. The generated vapour will be mixed with filtered, compressed air and ducted into the exposure chamber.

Test Atmosphere Characterisation: Prior to the start of the study a test material atmosphere will be generated within the exposure chamber. During this period airflow settings, test material in-put and, if necessary, the generating system will be varied to achieve the required atmospheric concentrations.

If the test material is heated, particulates will be measured in the chamber by cascade impactor to determine whether an aerosol is present due to condensation.

Pre-Study Sighting: During characterisation individual rats will be exposed to varying atmosphere concentrations of the vapour material and monitored for severe adverse effects. The target concentration for the main study will be determined based on the results of the sighting work.

7. **EXPOSURE MONITORING**

Chamber Concentrations: Nominal concentrations will be calculated by dividing the total weight of test material disseminated into the chamber atmosphere by the total volume of air passed through the exposure chamber.

The actual concentration of the vapour will be determined by chemical analysis approximately ½, 1, 2, 3 and 4 hours after the start of the exposure period. Sampling will be performed from a point in the chamber representative of that occupied by the external nares of the test animals (i.e. in the animals' breathing zone).

Monitoring of Air Flow: The air flow through the exposure chamber will be measured at least every thirty minutes throughout the exposure period.

APPENDIX VIII (continued)

Exposure Chamber Temperature & Relative Humidity: The temperature and relative humidity inside the exposure chamber will be measured by an electronic digital recorder located in a vacant port in the animals' breathing zone and recorded every thirty minutes throughout the exposure.

Oxygen Content of the Chamber: Oxygen levels within the exposure chamber will be monitored by an electronic digital oxygen analyser, as appropriate, to ensure that the chamber oxygen concentration remains above 19%.

8. OBSERVATIONS

Clinical Signs: Thirty minutes, one, two and three hours during exposure, immediately on removal from the chamber and one hour after completion of exposure, then at least once daily for fourteen days. The onset, intensity and duration of any signs observed will be recorded. The observation period may be extended if signs of toxicity are persistent at Day 14.

Throughout the study animals may be killed *in extremis* in order to reduce pain or suffering. The decision to kill any animal will be made by the Study Director.

Bodyweight: Prior to treatment on the day of exposure and on Days 7 and 14 or at death.

Necropsy: Performed on all animals dying or killed in extremis during the study and on all survivors killed by intravenous injection of sodium pentobarbitone. Whole body necropsies will be performed with special attention to the lungs and upper respiratory tract for signs of irritancy or local toxicity.

Preservation and fixation of tissues for subsequent histopathological examination will only be undertaken at the specific request of, and at extra cost to, the sponsor.

9. EVALUATION OF DATA

The inhalation LC_{50} (4 hour exposure and, if possible 1 hour exposure) will be calculated by an accepted method eg. Weil (1952), Litchfield and Wilcoxon (1949), Finney (1971). Where possible separate LC_{50} values and 95% confidence limits will be calculated for males and females separately. Clinical observations, necropsy and bodyweight records will be examined for treatment-related effects.

APPENDIX VIII (continued)

10. QUALITY ASSURANCE

The final report will be audited by Safepharm Quality Assurance Unit, in accordance with QAU Standard Operating Procedures. The routine inspection of short term toxicity studies is carried out as a continuous process designed to encompass all major phases of each study type once a month.

11. FINAL REPORT

The final report will include the following information:

Summary report.
Study design and test system justification.
Test material description, identification and storage conditions.
Animals and animal husbandry: Species, strain, source, environmental conditions, diet, etc.

Atmosphere generation: Description of apparatus including; chamber design and type, method of conditioning air, method of animal restraint, equipment for measuring temperature, humidity, oxygen concentration, test atmosphere concentration and environmental conditions.

Observations: Mortality, clinical signs during exposure and for the duration of the study, bodyweights, necropsy findings and, if requested by the Sponsor, any histopathological findings.

Evaluation of data.
LC_{50} value for each sex with 95% confidence limits and method of calculation (if applicable).
Conclusion.
Schematic diagram of exposure system used.
Tabulation of data including:
Atmosphere concentrations (actual and nominal), airflow rates, equilibration period, mortality, individual clinical observations, individual bodyweights, individual necropsy findings and environmental conditions within the exposure chamber.
Method of chemical analysis.

13. ARCHIVE

Unless instructed otherwise by the Sponsor, all original data and the final report will be retained in the Safepharm archive for a period of five years. At the end of this period, the sponsor's instructions will be sought.